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EXAMINER

LONG, SCOTT

ART UNIT

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PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 10/546,139	Applicant(s) CHATEAU ET AL.	
	Examiner SCOTT LONG	Art Unit 1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 17 December 2009.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 13, 14 and 44-49 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 13, 14 and 44-49 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☒ All b) ☐ Some * c) ☐ None of:
1. ☒ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____.
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|---|---|
| 1) <input type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) |
| 2) <input type="checkbox"/> Notice of Draftperson's Patent Drawing Review (PTO-948) | Paper No(s)/Mail Date. _____ |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

The examiner acknowledges receipt of Applicant's Remarks and Claim amendments, filed on 14 December 2009.

Claim Status

Claims 13-14 and 44-49 are pending. Claims 13 and 48 are amended. Claims 1-12 and 15-42 are cancelled. Claims 13-14 and 44-49 are under current examination.

Priority

This application claims benefit as a 371 of PCT/FR04/00354 (filed 02/17/2004), which claims foreign priority from foreign patent applications, FRANCE 03/13054 (filed 11/6/2003) and FRANCE 03/05769 (filed 5/14/2003) and FRANCE 03/05768 (filed 5/14/2003) and FRANCE 03/01924 (filed 2/18/2003). The instant application has been granted the benefit date, 18 February 2003, from the application FRANCE 03/01924.

RESPONSE TO ARGUMENTS

35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person

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having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 13-14 and 44-49 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Richaud et al. (J. Biological Chemistry. December 25, 1993; 268(36):26827-26835) in view of Schroder et al. (US2008/0118959) and further in view of Thanbichler et al. (Journal of Bacteriology. Jan 1999; 181(2): 662-665) for the reasons of record and the comments below.

The applicant's arguments have been fully considered but are unpersuasive.

The applicant's remarks argue that the cited art, whether alone or in combination fail to disclose the features of claim 13 (Remarks, page 5, 1st parag).

The applicant's remarks particularly argue that the cited art fails to teach "cultivating the modified microorganism on a minimal medium containing no methionine, S-adenosylmethionine, homocysteine, or cystathionine in the presence of methylmercaptan" (Remarks, page 5, parag.2). Contrary to the applicant's assertion, Richaud teaches cultivating a modified strain of E.coli (identified by the name, β 254) in MS minimal medium without amino acids added to the growth medium and particularly indicates that L-Cystathionine and L-Homocysteine are not present (Table III and page 26831, col.1, lines 8-11). While, Richaud teach magnesium sulfate as a sulfur source in their method and does not utilize methylmercaptans in their method as a source of sulfur, Schroeder et al. teach that a variety of sulfur-containing compounds, including sulfates and mercaptans, can be used as a sulfur source in methods of producing molecules such as methionine (parag. 0235). Therefore, it is obvious to a skilled artisan to substitute a mercaptan, such as methylmercaptan, for the magnesium sulfate in the method of Richaud. Accordingly, the Office deems the cited art as teaching or suggesting these claim limitations or as an obvious equivalent substitution to a skilled artisan . Therefore, the examiner finds the applicant's argument unpersuasive.

The applicant's remarks argue (1) Richaud makes use of a strain which was modified before being cultured...There is no such modification of the modified strain in the method of the invention. The examiner interprets the instant claims differently from the implication suggested in Remarks, page 6, parag.1. The instant claims clearly

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indicate "disrupting metE gene in an initial microorganism to yield a modified microorganism, wherein...growth is impaired." Richaud teach disrupting metC gene in an initial microorganism to yield a modified microorganism, wherein growth is impaired. Therefore, the cited art suggests a very similar method. As for the applicant's remarks regarding the introduction of a plasmid into the cells of Richard which can over express the metB gene, this would be outside the scope of the instant claims, because the instant claims use open language to describe the claimed invention. Therefore, the examiner finds the applicant's argument unpersuasive.

The applicant objects to the use of Schroder in the obviousness rejection, stating that Schroder does not add anything that would remedy the aforementioned deficiencies of Richaud. Contrary to the applicant's opinion, the examiner views Schroder as demonstrating that mercaptans can be substituted for magnesium sulfate as a sulfur source in Richaud. Furthermore, Schroder et al. makes a clearer case for disrupting metE, as described in the pending rejection. Therefore, the examiner finds the applicant's argument unpersuasive.

The applicant's remarks (page 6, last paragraph) argue that Schroder's disclosure of disruption of the metK gene is not a deletion of metK gene. The specification does not limit "disruption" to deletion mutations. Therefore, the examiner concludes the applicant is arguing limitations which are not in the instant claims. Therefore, the examiner finds the applicant's argument unpersuasive.

The applicant's remarks state that the cited art does not teach culturing the modified microorganism for multiple generations under selection pressure (page 7,

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parag.1). Contrary to the applicant's view, Richaud teach culturing the mutant microorganism under selection pressure. As most *E. coli* divide about every 20 minutes, the examiner concludes a skilled artisan would understand that multiple generations of the modified microorganism have been cultured under selection pressure. Therefore, the examiner finds the applicant's argument unpersuasive.

The applicant objects to the use of Thanbichler et al. in the obviousness rejection, stating that Thanbichler does not add anything that would remedy the aforementioned deficiencies of Richaud and Schroder (Remarks, page 7, parags. 2-4). In particular, the applicant argues that the deletion of metE gene in Thanbichler is irrelevant in relation to the claimed invention because the deletion of metE gene in Thanbichler was not done to allow the strain to evolve a metabolic pathway to compensate for impaired growth. The examiner acknowledges that the rationale behind Thanbichler's research was not to devise an applied method like that of the instant claims. Rather, the nature of Thanbichler's work is basic research, in which they are using microbial genetics and modern molecular biology techniques to understand the enzymes involved in methionine metabolism. In this respect, the work of Thanbichler is relevant to the instant obviousness rejection, because, in the words of Thanbichler, the "results described here identify the function of two unassigned open reading frames from *E. coli*. Their products are involved in the uptake of S-methylmethionine and in the methyl transfer to homocysteine, rendering two molecules of methionine" (page 664, col.1, last parag.). Furthermore, the teachings of Thanbichler et al. suggest that naturally occurring microorganism strains having mutations in the metE gene have

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evolved proteins that can compensate for disruptions to metE. While not disclosing a method of directed evolution, Thanbichler et al. disclose the importance of the metE gene in methionine metabolism and further disclose the general interest (i.e., motivation) in the art for studying mutations to the metE gene. Accordingly, the examiner finds that the Thanbichler is not completely irrelevant to the pending rejection. Therefore, the examiner finds the applicant's argument unpersuasive.

Therefore, the examiner hereby maintains the rejection of claims 13-14 and 44-49 under 35 U.S.C. 103(a) as being unpatentable over Richaud et al. in view of Schroder et al. and further in view of Thanbichler et al.

The examiner reiterates the pending rejection:

Claims 13-14 and 44-49 are rejected under 35 U.S.C. 103(a) as being unpatentable over Richaud et al. (J. Biological Chemistry. December 25, 1993; 268(36):26827-26835) in view of Schroder et al. (US2008/0118959) and further in view of Thanbichler et al. (Journal of Bacteriology. Jan 1999; 181(2): 662-665).

Claim 13 is directed to a method for the producing an evolved protein involved in methionine biosynthesis pathway, the method comprising: a) disrupting metE gene in an initial microorganism to yield a modified microorganism, wherein the ability of the modified microorganism to grow is impaired when the modified microorganism is grown on a minimal medium containing no methionine, S-adenosylmethionine, homocysteine, or cystathionine; b) culturing the modified microorganism obtained in step (a) on said minimal medium containing no methionine, S-adenosylmethionine, homocysteine, or cystathionine for multiple generations, under selection pressure in the presence of

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methylmercaptan, allowing the modified microorganism to evolve a metabolic pathway; c) selecting an evolved microorganism from step (b) able to grow on said minimal medium comprising methylmercaptan and containing no methionine, S-adenosylmethionine, homocysteine, or cystathionine, wherein at least one protein has evolved in the methionine biosynthesis pathway allowing the modified microorganism to produce methionine and proliferate; d) isolating the evolved protein.

The specification defines an evolved protein as “a sequence of amino acids (protein sequence) that differs in at least one amino acid from the initial protein sequence after selection” (page 4, lines 5-8). According to the specification, selection is defined as “a culture method used to select microorganisms that have evolved in such a way that a modification does not affect growth anymore” (page 3, lines 22-24). The specification does not explicitly define the phrase “directed genetic modification.” Accordingly, the examiner will interpret these terms broadly.

Richaud et al. broadly teach a method for producing an evolved protein involved in methionine biosynthesis pathway, comprising most of the steps set forth in the instant claims, except that rather than disrupting the *metE* gene, as in the instant claims, Richaud et al. disrupt the *metC* gene. Accordingly, Richaud and the instant claims are both species of a broader genus of methods of modifying genes of the methionine biosynthesis pathway, using a protein evolution strategy. The details of Richaud are elaborated below.

In addition, Schroder et al. teach a method for producing sulfur-containing fine chemicals, such as methionine, comprising most of the steps set forth in the instant

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claims, except that rather than disrupting the *metE* gene, as in the instant claims, Schroder et al. disrupt the *metK* gene. Furthermore, Schroder et al. teach that the *metE* gene is an important enzyme involved in the synthesis of methionine which can be studied during the method of producing methionine (parag.0009). The teachings of Schroder et al. are elaborated below.

Richaud et al. teach a very similar method as that of the instant claims. Richaud et al. teach “disrupting the *metC* gene” (abstract) of *E. coli*, which the examiner interprets as suggesting a generalizable method directed to “generating a directed genetic modification in a gene of interest in an initial microorganism,” of which *metE* would be such a gene that could be employed in part a) of claim 13. Richaud et al. teach “a latent metabolite could under certain circumstances fulfill an essential need in cell chemistry, the way would be open for establishing a biosynthetic pathway *de novo*” (page 26827, col.1), which suggests a generalizable method step directed to evolution of a metabolic pathway of which the limitations of part b) claim 13 are but one embodiment. Richaud et al. teach culturing the mutant microorganism in minimal medium. Richaud et al. also teach “techniques of metabolic engineering can be applied to evolving the chemical constitution of living cells beyond its present state” (abstract), which is similar to the broad outline of the instant invention provided by the specification. Furthermore, Richaud et al. teach “a *metC* mutation enhances the growth of *dap* strains exogenously supplied with L-lanthionine, meso-lanthionine, or L-allo-cystathionine as the cross-linking amino acid' and is absolutely required for growing such strains with exogenous L-cystathionine (Table VI). The broad activity of

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cystathionase, which is indeed known to degrade generically L-cysteine thioethers *in vitro*, can thus be rationalized as fulfilling a corrective task, which adds to the biosynthetic function of the enzyme in *E. coli* metabolism ” (page 26834, col.1, parag.1), which the examiner interprets as suggesting a general methodology similar to the limitations of part a) and b) of claim 13, where the production or consumption of a substrate is inhibited when the modified microorganism is grown on a defined medium and wherein the ability of the modified microorganisms to grow is impaired and wherein the defined medium can contain a co-substrate. Richaud et al. further indicate, “[t]hese strains can thus be viewed as having undergone an evolutionary commitment to use cysteine thioethers for building their cell wall. Although this commitment did not result from natural selection but was rationally set up in their genome, the fitness of the committed strains might now be improved by natural selection” (page 26834, col.2, parag.1). Therefore, Richaud teaches a method of for producing an evolved microorganism having an evolved protein involved in the methionine biosynthesis pathway, by disrupting the *metC* gene. Accordingly, the examiner concludes that a skilled artisan would be able to generalize this method to apply it to other proteins involved in the methionine biosynthesis pathway, such as by disrupting genes including the *metE* gene.

Richaud et al. do not teach: (1) disruption of *metE*, (2) isolation of the evolved protein and (3) growing the microorganism in minimal medium with methylmercaptan as a sulfur source.

The instant claims share a basic scheme common to classical microbial genetics. Schroder et al. teach the basic idea used in the instant claims: “mutagenesis, selection, and choice of mutants” (parag.0004). Schroder et al. teach deletion of *metK* gene in bacteria. Schroder teach culturing and fermentation of a methionine-producing microorganism with a reduced metK activity (parag.0071). Schroder teach growing the microorganisms in minimal medium with mercaptans as a sulfur source (parag.0235) for multiple generations (parag.0229). Schroder et al. teach protein extracts prepared from cultured cells. Finally, Schroder et al suggest that metE and closely related metH are important enzymes involved in the synthesis of methionine which can be studied during the method of producing methionine.

Richaud teaches cultivating a modified strain of E.coli (identified by the name, β 254) in MS minimal medium without amino acids added to the growth medium and particularly indicates that L-Cystathionine and L-Homocysteine are not present (Table III and page 26831, col.1, lines 8-11), thereby satisfying the limitations directed to growing the microorganism in minimal medium containing no methionine, S-adenosylmethionine, homocysteine, or cystathionine.

While, Richaud teach magnesium sulfate as a sulfur source in their method and does not utilize methylmercaptans in their method as a source of sulfur, Schroeder et al. teach that a variety of sulfur-containing compounds, including sulfates and mercaptans, can be used as a sulfur source in methods of producing molecules such as methionine (parag. 0235). Therefore, it is obvious to a skilled artisan to substitute a mercaptan, such as methylmercaptan, for the magnesium sulfate in the method of

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Richaud. Accordingly, the Office deems the cited art as teaching or suggesting these claim limitations or as an obvious equivalent substitution to a skilled artisan .

Schroder et al. do not explicitly teach disruption of *metE*.

Thanbichler et al. teach the importance of *metE* and *metH* in the synthesis of methionine. Furthermore, Thanbichler et al. teach studying an *E. coli* mutant having a deletion of the *metE* gene for its involvement in alternative pathways of producing methionine. The deletion of *metE* gene in Thanbichler's research was not performed to allow the strain to evolve a metabolic pathway to compensate for impaired growth. The examiner acknowledges that the rationale behind Thanbichler's research was not to devise an applied method like that of the instant claims. Rather, the nature of Thanbichler's work is basic research, in which they are using microbial genetics and modern molecular biology techniques to understand the enzymes involved in methionine metabolism. In this respect, the work of Thanbichler is relevant to the instant obviousness rejection, because, in the words of Thanbichler, the "results described here identify the function of two unassigned open reading frames from *E. coli*. Their products are involved in the uptake of S-methylmethionine and in the methyl transfer to homocysteine, rendering two molecules of methionine" (page 664, col.1, last parag.). Furthermore, the teachings of Thanbichler et al. suggest that naturally occurring microorganism strains having mutations in the *metE* gene have evolved proteins that can compensate for disruptions to *metE*. While not disclosing a method of directed evolution, Thanbichler et al. disclose the importance of the *metE* gene in

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methionine metabolism and further disclose the general interest (i.e., motivation) in the art for studying mutations to the metE gene.

Richaud et al. and Schroder et al. teach the basic concepts of claims 44-49.

Claim 44 is directed to the method of claim 13, wherein the genetic modification comprises the directed mutation or deletion of a gene of interest or the directed modification of a promoter in the gene of interest. Richaud et al. teach “disrupting the metC gene” (abstract) of *E. coli*, which the examiner interprets as satisfying the limitations directed to “generating a directed genetic modification in a gene of interest in an initial microorganism,” as described in part a) of claim 13. Likewise, Schroder et al. teach deletion of a gene involved in methionine synthesis, metK.

Claim 45 is directed to the method of claim 13, wherein the genetic modification consists in the removal of most of the gene of interest. Richaud et al. teach “disrupting the metC gene” (abstract) of *E. coli*, which the examiner interprets as satisfying the limitations directed to “generating a directed genetic modification in a gene of interest in an initial microorganism,” as described in part a) of claim 13. The type of mutation does not seem to be particularly important to the practice of the method. Any type of null mutant, whether created by a deletion, point mutation, etc would be obvious in light of the teachings of Richaud et al. Likewise, Schroder et al. teach deletion of a gene involved in methionine synthesis, metK.

Claim 46 is directed to the method of claim 13, wherein the gene of interest is replaced with a selection marker gene. The type of mutation does not seem to be particularly important to the practice of the method. Any type of null mutant, whether

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created by a knockout by replacing the gene of interest with a selection marker, or by any other known means, would be obvious in light of the teachings of Richaud et al.

Claim 47 is directed to the method of claim 13, wherein the microorganism is a bacteria. Richaud et al. teach a method which uses *E. coli*. Schroder et al. and Thanbichler et al. teach bacteria.

Claim 48 is directed to the method of claim 13, wherein the microorganism is a *Escherichia spp.* Richaud et al. teach a method which uses *Escherichia coli*. Schroder and Thanbichler teach *Escherichia coli*.

Claim 49 is directed to the method of claim 13, wherein the microorganism is *E. coli* and *C. glutamicum*. The instant specification does not describe a method that uses two different microorganisms, so the examiner is interpreting the instant claim as reciting “or” rather than “and.” In particular, the specification describes using either *E. coli* or *C. glutamicum* on page 8, lines 8-10 of the specification. Richaud et al. and Schroder et al. and Thanbichler et al. teach methods which use *E. coli*.

It would have been obvious to the person of ordinary skill in the art at the time the invention was made to combine the teachings of Richaud et al and Shroder et al. and Thanbichler et al. so that the *metE* gene is disrupted and a method of protein evolution is practiced such that an evolved protein produced by the microorganisms of can be isolated and methionine is produced.

Regarding the rationale for combining prior art elements according to known methods to yield predictable results, all of the claimed elements were known in the prior art and one skilled in the art could have combined the element as claimed by known

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methods with no change in their respective functions, and the combination would have yielded predictable results to one of ordinary skill in the art at the time of the invention. Each of the elements (methods of protein evolution; importance of *metE* in methionine biosynthesis) is taught by Richaud or Shroder or Thanbichler and further they are taught in various combinations and are shown to be immunogenic or used as vaccines. It would be therefore predictably obvious to use a combination of these elements in a method of producing an evolved protein during methionine biosynthesis. The combined teachings suggesting substituting *metE* in the methods of Richard and Schroder.

The skilled artisan would have had a reasonable expectation of success in combining the teachings of Richaud et al. and Shroder et al. and Thanbichler et al. because these teachings generated evolved microorganisms and discuss the proteins which make possible the growth of the auxotrophic organisms.

Therefore the method as taught by Richaud et al. in view of Shroder et al. and further in view of Thanbichler would have been *prima facie* obvious over the method of the instant application.

Conclusion

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

No claims allowed.

Examiner Contact Information

Any inquiry concerning this communication or earlier communications from the examiner should be directed to **Scott Long** whose telephone number is **571-272-9048**. The examiner can normally be reached on Monday - Friday, 9am - 5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Joseph Woitach** can be reached on **571-272-0739**. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Scott Long/
Examiner, Art Unit 1633